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Giant unilamellar vesicle formation under physiologically relevant conditions

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ABSTRACT

We present an upgrade to the giant unilamellar vesicle (GUV) electroformation method allowing easy GUV production in different buffers and with various membrane compositions. Our experimental results reveal that lipid deposits obtained from aqueous liposome or proteoliposome dispersions are highly efficient for GUV electroformation. This is related to the ability of such dispersions to produce readily well-oriented membrane stacks. Furthermore, we present a protocol for GUV electroformation in various aqueous media, including electrolyte-containing buffers at characteristic concentrations of biological fluids. This work unlocks historical barriers to GUV applications in scientific fields like biology, biochemistry, or biophysics where membrane composition, as well as its aqueous environment, should be adapted to biological significance.

1. Introduction

Giant unilamellar vesicles (GUVs) are definitely a fascinating model system (Luisi and Walde, 2000). They are useful for physicists and biophysicists as model systems (Ambroggio et al., 2005; Dimova et al., 2006; Staneva et al., 2005) or as passive objects for method developments (Baumgart et al., 2003; Korlach et al., 2005; Tokumasu et al., 2003). The number of published studies referring to GUV has increased dramatically during the last few years, probably due to GUVs direct and easy observation by optical microscopy. Indeed, to broaden their use for biochemistry, biology or any other field, membrane composition and aqueous environments should be readily adaptable. Yet, this is not hitherto the case due to a lack of an experimental protocol to produce GUVs under the physiologically relevant pH and salinity.

Currently, the protocols for GUV preparation always follow a two-step procedure. The first step is the deposit of some phospholipid on a substrate, the choice of substrate being dictated by the second step, i.e., GUV formation from the dry lipid deposit. In this second step, either slow spontaneous swelling, or the much faster process of electroformation is used. Electroformation is usually preferred for two reasons: first because of its speed, an important factor when working with biomolecules, and second because of the better quality of the GUVs. Spontaneous swelling is used for physiologically relevant conditions due to the widely held belief that electroformation does not work in the presence of salt (for a recent review on the subject, see (Dimova et al., 2006)).

The formation of the lipid deposit on the electrodes, and its subsequent quality, is crucial for the successful production of GUVs by electroformation. Deposits are generally made from a phospholipid solution in organic solvents (Angelova et al., 1992; Dimova et al., 2006; Evans and Needham, 1986), drastically limiting the complexity of GUV membrane composition. Yet, aqueous liposome suspensions can be made from complex lipid and protein mixtures. Initial work by our group using aqueous vesicle suspensions as deposits showed that GUV electroformation rates were high (Valverde, 2000). This approach was applied by Girard et al. (2004) following our suggestions. It was then generalized by us to a variety of liposome preparations. Herein we present the corresponding method for successful GUV production from aqueous deposits, as well as a procedure for the electroformation of GUVs in different buffer environments, including physiologically relevant conditions.

2. Materials and methods

Natural and synthetic phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL, USA), namely egg and soy Diacylglycero Phosphatidylcholine (eggPC, soyPC), stearoyl-oleoyl phosphatidylcholine (SOPC) and palmitoyl-oleoyl phosphatidylcholine (POPC). Bacteriorhodopsin (BR) from *H. salinarium* purple membranes was kindly provided by J.-L. Rigaud and D. Lévy (UMR CNRS 168, Institut Curie, Paris). As a hydrophobic molecule with a poor solubility in major organic solvents, we used the 2-photons fluorescent dye 4,4,-[(9,9-dinonyl-9H-fluorene-2,7-diyl)bis[(1E)

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-2,1-ethenediyl-4,1-phenylene-(1E)-2,1-ethenediyl]]dibenzenamine called NH2-PV2-FL in the following and produced by M. Blanchard-Desce's team from our laboratory.

Buffer solutions at 1–10 mmol/L Tris (Trizma Base, Sigma–Aldrich Chimie, France) and 0.2–2 mmol/L EDTA (ethylenediaminetetraacetic acid, Sigma–Aldrich Chimie, France) adjusted to pH 7.4 were used. Ionic strength was changed using NaCl (Sigma–Aldrich Chimie, France) from 0 up to 250 mmol/L (osmolarity was measured by cryoscopy using an Osmomat 030 (Gonotec, Germany), and varied from ~20 up to ~500 mOsmol/L). Glucose or sucrose (Sigma–Aldrich Chimie, France) solutions were also prepared at 200 mmol/L.

Small unilamellar vesicles (SUV), obtained by sonication, and large unilamellar vesicles (LUV), obtained by extrusion or reverse phase evaporation (REV), were produced according to standard procedures and used directly.

The electroformation cell was already presented in (Méléard et al., 1997). Platinum wire with a 0.8 mm diameter was obtained from Goodfellow (Huntington, England). The cell itself was purchased from Hellma-France (Paris) as standard optical glass cuvette with 1 mm inner thickness. The two platinum electrodes were held at about 3 mm axis-to-axis. They were connected to a function generator (3324A, Hewlett-Packard, Boeblingen, Germany) to change voltage difference and frequency depending on the GUV swelling milieu. Observations were made using an inverted microscope (Axiovert 135, Zeiss, Germany), equipped with a 63× objective for phase contrast and fluorescence and a 20× objective for polarized light. A CCD video camera (C5985, Hamamatsu, Japan) connected to a computer for image capture was used.

Small angle X-ray scattering experiments were carried out in the laboratory using a rotating Cu anode X-ray generator (Bruker Nonius FR591) equipped with a 2D reflection system (Montel 200 multilayer graded optics) used as a monochromator and collimator (K_α wavelength $\lambda = 1.54187$ Å). Three sets of vertical and horizontal slits were used leading to a spot size of 0.5 mm × 0.5 mm. Diffraction was collected on a 2D MarResearch imaging plate detector (mar345) with a plate diameter of 345 mm and a pixel size of 150 μm × 150 μm. Oriented samples were obtained by pipetting small volumes of aqueous lipid dispersions on one of the platinum electrodes, used for the electroformation procedure presented below, or on a glass plate, and directly placed in the X-ray beam. Calibration of the sample-to-detector distance was achieved using Quartz α powder and was 283 mm.

3. Results and discussion

3.1. GUV formation from aqueous vesicle deposits

When using deionized water or a low concentration buffer (less than 10 mmol/L for total salt concentration) as an electroformation medium, it should be noted that one can produce giant vesicles from small-size liposomes prepared according to standard protocols (sonication, extrusion, REV, etc.). The SUVs or LUVs are diluted to a concentration suitable for the GUV formation process, which is typically about 0.25 mg/mL. These freshly prepared water-based lipid dispersions (SUV, LUV by extrusion or REV, etc.) are then placed on the platinum electrodes as small drops not exceeding 2 μL (on average, four deposits per electrode). The small volume of each aqueous deposit is necessary to minimize lateral displacement or dropping, leading to a very small average bilayer number per spot (between 10 and 50) after evaporation of excess water. This drying of deposits can be performed at room temperature using ambient air or be accelerated by placing the electrodes and their lipid dispersion drops in a desiccator at reduced pressure. Alternatively, deposits can be partially dehydrated using equilibration with an aqueous polymer solution (e.g., dextran or PEG) or with a saturated salt solution (e.g., NaCl). At equilibrium, water activity (humidity) is then fixed by the osmotic pressure of the polymer or the salt solutions. This should be useful for membranes for which individual compounds (e.g., protein-containing membranes) destabilize at very low water activity. To limit oxidization, partial or complete dehydration may be performed using a desiccator filled with either argon or nitrogen. Exposition to direct sunlight should be avoided.

Eventually, by filling the electroformation cell with water or a low concentration buffer, one can see smooth lipid deposits on the electrodes whatever the type of aqueous vesicles used. It should be noted that the filling of the electroformation cell should be done very carefully in order to keep the lipid films on the electrodes. For example, systematic delamination was observed during cell filling when phospholipid films, hydrated at 100% relative humidity (room temperature), were used. On the contrary, lowering the hydration of the lipids to an equivalent of 75% relative humidity (room temperature) was sufficient to keep the film on the electrodes.

The process of electroformation starts as soon as the electroformation cell is filled with the desired medium to avoid any spontaneous swelling. Generally speaking, GUV electroformation involves three stages, each of which is controlled by changes of the applied oscillating electric field (Table 1): the first step, lasting a time t, corresponds to an increase of the field amplitude E up to a maximum value E_{max} at fixed frequency f; the second to the swelling period during which electric field parameters (E_{max} and f) are constant; and the third to the "rebounding" period. During the first step the GUVs gradually start to form from the deposits on the electrodes. Typically within 20–30 min grapes of GUVs can be noticed increasing progressively in size. To control this step, the electric field has to be strong enough to maintain a sphere-like shape for the growing vesicles. The second step, the swelling period, is optional and may be omitted at low salinity when the electric field amplitude increases slowly to its maximum value, E_{max}. Otherwise, this swelling period is essential in order to control the final size of the GUVs. For instance, if E_{max} is reached very quickly, the GUVs might be quite small (from 5 to 10 μm) and it may be beneficial to include this swelling period in order to adapt the vesicle size to the desired purpose (e.g., objective magnification, micromanipulation set-up, etc.). During the "rebounding" period, the final step

Table 1
Electroformation parameters in low and high salinity buffers at pH 7.4

	Step	E (V/m)	f (Hz)	t (min)
Low salinity	1	50–700	10	60–90
	2	700	10	0–60
	3	700	10–4	30–60
High salinity	1	50–1300	500	30
	2	1300	500	90
	3	1300	500–50	30–60

E is the alternating electric field amplitude (peak to peak) applied during one of the steps, f its frequency and t is the step duration. The arrow indicates a slow change of the corresponding parameter during the electroformation swelling.